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Sequence Requirements of Spider Silk Elastic Proteins

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13. ABSTRACT (Maximum 200 words)

Sequence for flagelliform silk proteins from *Nephila*, *Argiope*, and *Araneus* species have been obtained. These sequences show signifiicant sequence differences although the changes are not predicted to affect the secondary structure of the protein and therefore its function will be the same. *Araneus* shows the presence of two proteins, one a flagelliform protein and the other a major ampullate silk protein. In addition we have cloned and sequenced a number of silk proteins from glands of undetermined evolutionary origin in new spider species. We have also cloned and substantially sequenced a new protein cDNA from aciniform glands whose silk is used for prey wrapping.

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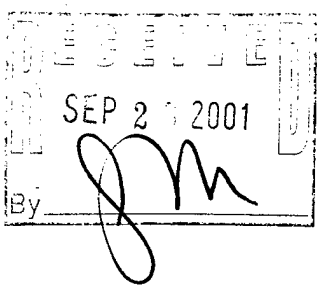

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Title: Sequence Requirements of Elastic Spider Silk Proteins

Grant Number: DAA-98-1-0262

Institution: University of Wyoming

Author of Report: Randolph V. Lewis

Manuscripts:

To be submitted:

(2001) Gatesy, J., Hayashi, C.Y., and Lewis, R.V. Codon Usage in Spider Silk Proteins from Diverse Species (to be submitted)

(2001) Jones, J.A. and Lewis, R.V. Mechanical and Structural Studies of Spider Silk Films (to be submitted)

(2001) Shipley, N.H. and Lewis, R.V. Structural Analysis of Flagelliform Silk Proteins (to be submitted)

Accepted and in press:

(2001) Hayashi, C.Y. Evolution of Spider Silk Proteins: Insight from Phylogenetic Analyses (In press, Molecular Techniques in Evolutionary Biology)

Published:

(2001) Hayashi, C.Y. and Lewis, R.V. My Favorite Molecule: Spider Silk Bioessays 23:750-756

(2001) Gatesy, J., Hayashi, C.Y., Motriuk, D. and Lewis, R.V. Extreme Diversity, Conservation, and Convergence of Spider Silk Fibroin Sequences, Science 291:2603-05

(2000) Hayashi, C.Y. and Lewis, R.V. Molecular Architecture Controls the Evolution of a Modular

Spider Silk Protein Gene. Science 287:1477-1479

(2000) Hinman, M., Jones, J. and Lewis, R.V. The Next Superfiber: Synthetic Spider Silk, Trends in Biotechnology 18:374-79

(1999) Hayashi, C.Y., Shipley, N.H. and Lewis, R.V., Hypotheses that Correlate the Sequence, Structure, and Mechanical Properties of Spider Silk Proteins. (International J. of Biological Macromolecules 24:271-275)

(1998) Hayashi, C. and Lewis, R.V. Spider Flagelliform Silk Proteins J. of Mol. Biol. 275:773-784

Thesis:

(2000) Jones, J.A., Mechanical and Structural Studies of Spider Silk Films (M.S., Thesis, U.W.)

(2000) Shipley, N.H., Structural Analysis of Flagelliform Silk Proteins (M.S., Thesis, U.W.)

Scientific Personnel and Degrees Awarded during this period:

Cheryl Hayashi, Research Fellow

Dagmara Motriuk, PhD student

Shane Nelson, PhD student

Congzhou Liu, M.S. student

Tony Contento, PhD Degree awarded

Justin Jones, M.S. Degree Awarded

Nicola Shipley, M.S. Degree Awarded

Chris Van Kirk, undergraduate student

Inventions:

An Extremely Elastic Spider Silk Protein and the DNA encoding thereof- Issued

Spider Silk Protein Encoding Nucleic Acids, Polypeptides, Antibodies and Methods of Use Thereof-Application submitted

Specific Aims:

Determine the sequence variations in various species for the very elastic Flagelliform spider silk which makes up the core of the catching spiral in the web.

Results:

In the JMB paper we published the first sequence of a spider silk flagelliform silk protein. There were several interesting new findings about that protein. The first was that it showed consistency with the elastic protein in spider major ampullate silk (MaSp 2) in having a GPGXY sequence. In fact, this protein had up to 7 times more of this sequence in a row than did MaSp 2. However, the X and Y amino acids were different than in MaSp 2 and there was a very specific order they followed in the repeating sequence. The protein had GGX repeats as well as a "spacer", a non-silk-like region which was highly conserved. So the entire repeat unit was about 300 amino acids.

The 1999 IJMB paper described our efforts to understand the factors involved in the high tensile strength and elasticity of the spider silks. We concluded that the poly-Ala and Gly-Ala regions known to form ß-sheet structures were responsible for the tensile strength and, in fact, the poly-Ala regions created greater tensile strength. The GPGXY regions form nano-type springs and are the elastic portion of the protein. The greater the number of these units the greater the elasticity.

In the Science paper published in 2000 we reported genomic sequences of Flag genes that encompass both 5′ and 3′ ends and substantial portions of the intervening repetitive region. We cloned Flag genes from genomic DNAs of *N. clavipes* (*N.c.*) and *N. madagascariensis* (*N.m.*). The resulting 36 kb of sequence (Genbank AF218621-AF218624), represent the most extensive DNAs known for any spider silk. In contrast to other spider silk genes, Flag is not encoded by a single enormous exon. Instead, the Flag gene is evenly divided into exonic and intronic regions. In all, the Flag locus is estimated to span 30 kb and contain thirteen exons. A key and unique finding from this

paper was that the introns are highly conserved, even to a greater extent than the exons. In addition the introns within a species are more highly related than the same intron in the closely related species. We hypothesized this might be needed to insure the very repetitive gene was able to replicate without calamitous unequal crossovers occurring.

We have completed sequencing of large Flag cDNA clones from an *Argiope* species that shows sequence variations from the *Nephila* species. However, the sequence changes appear unlikely to alter the secondary and tertiary structure we predicted for the protein. This data will be published at a later date with other species data.

We are in the process of sequencing cDNAs from *Araneus* with interesting results. It was reported by Gosline that a major ampullate silk protein was also present in the flagelliform gland of *Araneus*. We could find no evidence for that in any of the *Nephila* or *Argiope* species. However, we have clear evidence that that protein is present in the flagelliform glands of *Araneus* with a typical flagelliform silk protein. The Flag protein is more similar to the *Argiope* protein than the *Nephila* protein as would be expected from the phylogenetic positions of the three species. A manuscript on these results will be submitted as soon as the sequencing is completed on the *Araneus* cDNAs.

Although not a specific aim of this grant the undergraduate student who was working on this project also created cDNA libraries from several other species who do not have flagelliform silk but have silk glands of unknown derivation. We have obtained cDNA sequence from many of those and they are part of the manuscript that was published in Science this year. These represent 8 new species and 17 new proteins. He has also very recently obtained sequence from glands from two new species. The unique finding described in this paper is that the MaSp proteins of the spiders making the typical orb web are quite highly conserved. In contrast those proteins from spiders using the silk for other purposes is very highly divergent. In fact, some have sequences showing almost no conservation with any other spider silk protein except at the very C-terminal end.

Even of more interest is that we have obtained the sequence of a silk cDNA from the aciniform glands which are used by the spider to wrap prey and is expected to be elastic as well. The sequence of this protein is substantially different from all known spider silk sequences, showing a nearly 200

amino acid long repeat sequence. The protein is very clearly an araneoid silk sequence based on the C-terminal non-repetitive region which is 80% identical to the sequence we and two other groups have obtained for this part of other silk proteins. This data forms the basis for our renewal application for this project.

Technology transfer:

A licensing agreement was signed between the University of Wyoming, WyoBiGen (a company started in 1994 by my group, based on the spider silk technology) and Nexia Biotechnology. We are actively collaborating with them to produce spider silk in goat's milk using our silk genes and their transfer technologies. In addition they are funding applied research both at WyoBiGen and at UW. We are providing Nexia with bacterially produced protein which is being used by the research group at Army Natick to generate methods to make fibers.

Other accomplishments:

Talks were given at three national meetings and two universities and to five service clubs, 6 elementary schools (along with spiders and videos), and the University Trustees (twice). We helped 5 high school students with science fair projects and had 7 minority high school students working in the laboratory for 8 weeks during the summer (one, as undergraduate, is now working in the laboratory on a research project). We also provided information and film for television documentaries from stations in the U.S., Canada, Britain and Germany.